

Effects of Repeated Administration of 3,4-methylenedioxymethamphetamine (MDMA) on Avoidance Memory and Cell Density in Rats' Hippocampus

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ABSTRACT

Introduction MDMA or ecstasy is a derivative of amphetamines used mostly by young people worldwide. Although the acute effects of this drug are known, the effect of chronic administration is not well studied. Therefore the aim of this study was to determine the effects of repeated (long term) administration of MDMA on rats' memory and their hippocampal cell density.

Method: Young adult male Wistar rats 200 ± 20 g served as subjects. The rats were randomly distributed into three MDMA treated groups (3×2.5 mg/kg, 3×5 mg/kg, 3×10 mg/kg) and one control-saline group. All animals received MDMA intraperitoneally (3h apart; a challenge) 7th day of every week for consecutive 4 weeks. Animals were trained before and were tested after injections for their memory status using the standard passive avoidance method. Finally, 24hr after the memory test, rats were sacrificed and after tissue operations, the hippocampal astrocytes and neurons were counted.

Results: results showed that the number of neurons in all experimental groups was lower than the control-saline group. The most decreased number of neurons was shown in 5 mg/kg MDMA group compared to control-saline in all the regions of hippocampus. Also we found that repeated administration of MDMA reduced the number of hippocampal astrocytes.

Discussion: It is concluded that repeated administration of MDMA can reduce density of neurons and astrocytes and this decrease is not dose dependence.

1. Introduction

3,4-methylenedioxymethamphetamine (MDMA) or ecstasy is one of the most abused drugs (Simantov, 2004) among the young people worldwide. Although MDMA is associated with positive feelings and euphoria (Nichols, 1986) it is also associated with many side effects such as hallucinations, depression, reduced learning and memory loss (Green, Mehan, Elliott, O'Shea, & Colado, 2003; Morton, 2005).

MDMA is a synthetic, psychoactive drug that is chemically similar to methamphetamine. MDMA in the adult and teenage brains induces neuro-adaptive changes in mono-aminergic and glutamatergic systems (Green et al., 2003; Kindlundh-Högberg, Schiöth, & Svenningsson, 2007; Kindlundh-Högberg, Svenningsson, & Schiöth, 2006). Furthermore, the target areas of MDMA in the brain includes the cerebral cortex and the hippocampus, which are important areas for learning and memory functions (De Letter et al., 2003).

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The effects of MDMA vary according to the dose and the frequency and duration of use (Kalant, 2001).

At the microscopic level, administration of MDMA inhibits mossy fiber activity in the dentate gyrus (Giorgi et al., 2006); a part of hippocampal formation which generates neurons throughout life (Fazeli, Gharravi, Ghafari, Jahanshahi, & Gholipour, 2008; Jahanshahi, Khoshnazar, Azami, & Heidari, 2011). Some studies on the animal models have shown that MDMA administration also impairs adult neurogenesis (Capilla-Gonzalez & Hernandez-Rabaza, 2011). It has been reported that MDMA can reduce the proliferation rate under some administration patterns (Cho et al., 2007).

Also previous studies have shown that the high-dose of MDMA leads to neurotoxic effects such as neuronal apoptosis, reduced neuronal viability, and mitochondrial damage (Jimenez et al., 2004). It has also been shown that MDMA induces apoptosis, oxidative stress, and increased transcription of the cellular stress factor c-jun in primary cell cultures from the embryonic rat cortex (Capela et al., 2006; Stumm et al., 1999).

However the mechanisms of neurotoxic effect of MDMA remain poorly understood (Azami et al., 2009).

Astrocyte hypertrophy can occur as a result of neuronal injury and can lead to enhanced expression of GFAP, which can be used as a marker of neuronal damage, detected by immunohistochemical analysis of brain slices. A few studies have reported an increase in GFAP expression in the context of MDMA-induced neurotoxicity (Ádori, Andó, Kovács, & Bagdy, 2006; O'Callaghan & Miller, 2002; Pubill et al., 2003), whereas others have not been able to show this effect (Orío et al., 2004; Straiko, Coolen, Zemlan, & Gudelsky, 2007; Wang, Baumann, Xu, & Rothman, 2004).

In this study, we assessed the effects of repeated administration of MDMA on passive avoidance memory and on neuron-astrocytes density in the hippocampal formation in male wistar rats.

2. Methods

2.1. Animals

8-week male Wistar rats (Pasteur Institute, Tehran, Iran) weight 200 ± 20 g at the initiation of the study, served as subjects. Rats were housed pair-wise in air conditioned rooms at $22 \pm 3^\circ\text{C}$ and a humidity of 53% under a reversed dark-light cycle. The rats were ran-

domly distributed into three MDMA-treated and sham groups. All animals received three intraperitoneal injections (3hr apart; a challenge) every 7th day of the week for 4 consecutive weeks. MDMA (-3,4- methylenedioxy-N-methamphetamine-HCl, Sigma Pharmaceutical) was dissolved in saline on the day of testing. During the treatment day the MDMA low-dose group received 3×2.5 mg/kg MDMA, the MDMA middle dose group received 3×5 mg/kg, the MDMA high-dose group received 3×10 mg/kg whereas the control-saline group received a vehicle of sterile 0.9% saline solution (1 ml/kg). Eight animals were used for each group.

The Golestan University of Medical Sciences Guidelines for the Care and Use of Animals in Research were followed.

2.2. Inhibitory Avoidance Apparatus

Step-through inhibitory avoidance apparatus consisted of two boxes of the same size ($20 \times 20 \times 30$ cm). In the middle of a dividing wall, a guillotine door (7.9 cm²) could be lifted manually. The walls and floor of one compartment consisted of white opaque resin and the walls of the other compartment were dark. Intermittent electric shocks (50 Hz, 3 s, 1.5 mA intensity) were delivered to the grid floor (3 mm in diameter and 1 cm intervals) of the dark compartment by an isolated stimulator (Azami et al., 2010).

2.3. Behavioral Procedures

Our previous study described passive avoidance as follows: All animals were allowed to habituate in the experimental room (with light and sound attenuated) for at least 30 min prior to the experiments. Then, each animal was gently placed in the brightly lit compartment of the apparatus; after 5 s the guillotine door was opened and the animal was allowed to enter the dark module (Azami et al., 2010).

The latency with which the animal entered the dark chamber was recorded. Animals that waited more than 120 s to enter the dark chamber were excluded from the experiment.

Once the animal entered with all four-paws to the next chamber, the guillotine door was closed and the rat was immediately withdrawn from the compartment. This trial was repeated after 30 min. As in the acquisition trial, after 5s the guillotine door was opened, and as soon as the animal entered the dark (shock) compartment the door was closed; and a foot shock (50 Hz, 1 mA and

3 s) was immediately delivered to the grid floor of the dark room. After 20 s, the rat was removed from the apparatus and placed temporarily into its home cage. Two minutes later, the animal was retested in the same way as in the previous trials; if the rat did not enter the dark compartment during 120 s, a successful acquisition of inhibitory avoidance response was recorded. Otherwise, when the rat entered the dark compartment (before 120 s) a second time, the door was closed and the animal received the shock again. After retesting, if the rat learned inhibitory avoidance response successfully, it was moved to the cage and 24h after the training received MDMA or saline (3 h apart; a challenge) every 7th day for 4 weeks (i.p.). On the test day (4 weeks after training) each animal was gently placed in the light compartment and after 5 s the door was opened and step through latency (sec) was recorded in the absence of electric foot shocks, as indicator of inhibitory avoidance behavior.

2.4. Histology

24 h after the testing sessions, rats were anaesthetized with chloroform, decapitated and their brains were removed from the skull and stored in paraformaldehyde (4%) for two weeks. Then, the brains were placed in tissue processor apparatus for tissue procedures. Following this session, samples of the brain were embedded in paraffin and kept in refrigerator. Then the brains were sliced coronally into 8 μ m sections (from Bregma -2.5 mm to -4.5 mm of the hippocampal formation) (Paxinos & Watson, 2007) with a rotary microtome (MK 1110) and the sections were stained with cresyl violet for neurons (Fig.1) and PTAH for astrocytes (Fig.2) in accordance with routine laboratory procedures (Bancroft, Stevens, & Turner, 2008). A photograph of each section was produced using an Olympus BX 51 microscope and a DP 12 digital camera under a magnification of 1000 for Dentate Gyrus (DG) area and 400 for other areas. To measure the area density of the

neurons and astrocytes, the images were transferred to a computer. Using OLYSIA Autobioreporter software, Olympus Co, appropriate grids were superimposed on the pictures and the cells were counted manually. To perform an unbiased measurement, the individual was double-blinded and only the cells with significant granule cell characteristics were counted (Emamian et al., 2010; Jahanshahi, Golalipour, & Afshar, 2009; Jahanshahi, Sadeghi, Hosseini, Naghdi, & Marjani, 2010; Jahanshahi, Sadeghi, Hosseini, & Nashdi, 2007).

2.5. Statistical Analysis

The data were expressed as mean \pm SD. The statistical analysis was performed using one and two way analysis of variance (ANOVA) using SPSS software. Post-hoc comparison of means was carried out with the Tukey test for multiple comparisons, when appropriate. The level of statistical significance was set at $P < 0.05$.

3. Results

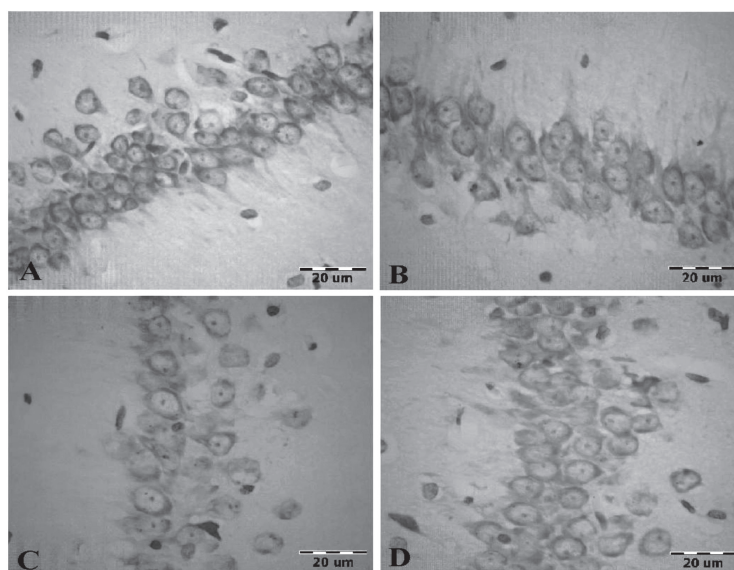
3.1. Avoidance Inhibitory Memory Test

The results of the memory task at the end of the 4th week for all groups are presented in table 1. As shown in table 1, the control-saline rats, which had not received MDMA, showed the lower latency entrance in the dark chamber after four weeks. In contrast, the other groups showed more latency compared the sham group. The most latency was observed in the 10 mg/kg group. We found significant difference between sham and experimented groups except the 5 mg/kg group ($P < 0.05$).

The mean of latency to entrance in dark chamber showed that MDMA at 10 mg/kg could be reminiscent of the shock in the dark chamber, while we did not found this phenomenon at the 5 mg/kg group.

Table 1. Table 1: Mean and SD of latency (Sec.) between control-saline and MDMA treated groups. A difference of $p \leq 0.05$ between the experimental groups was considered statistically significant. The 10 mg/kg group has the highest latency.

Groups	Mean (Sec.).	Std. Deviation	P value
Control-saline	68.75	4.633	
MDMA 2.5 mg/kg	190.85	12.865	0.033
MDMA 5 mg/kg	160.87	5.489	0.073
MDMA 10 mg/kg	267.28	14.096	0.002

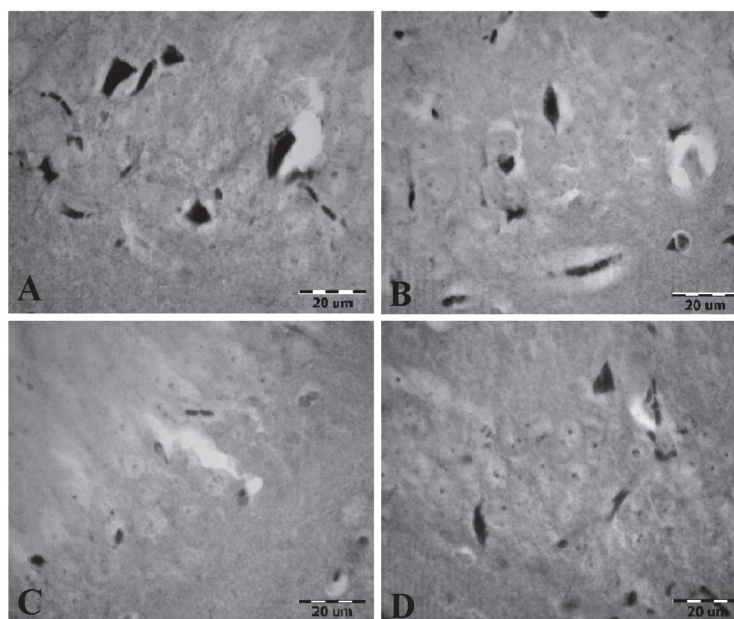


NEURSCIENCE

Figure 1. Photographs of coronal sections throughout the CA1 area of hippocampus in all groups (Cresyl violet staining $\times 40$, neurons are blue)

A: Control-saline B: MDMA 2.5 mg/kg C: MDMA 5 mg/kg D: MDMA 10 mg/kg

The highest decrease in the number of neurons was shown in response to MDMA with the dose of 5 mg/kg compared to the control-saline



NEURSCIENCE

Figure 2. Photographs of coronal sections throughout the CA1 area of hippocampus in all groups (PTAH staining $\times 40$, Astrocytes are purple)

A: Control-saline B: MDMA 2.5 mg/kg C: MDMA 5 mg/kg D: MDMA 10 mg/kg

The highest decrease in astrocytes number was shown in response to 5 mg/kg dose of MDMA compared to the control-saline

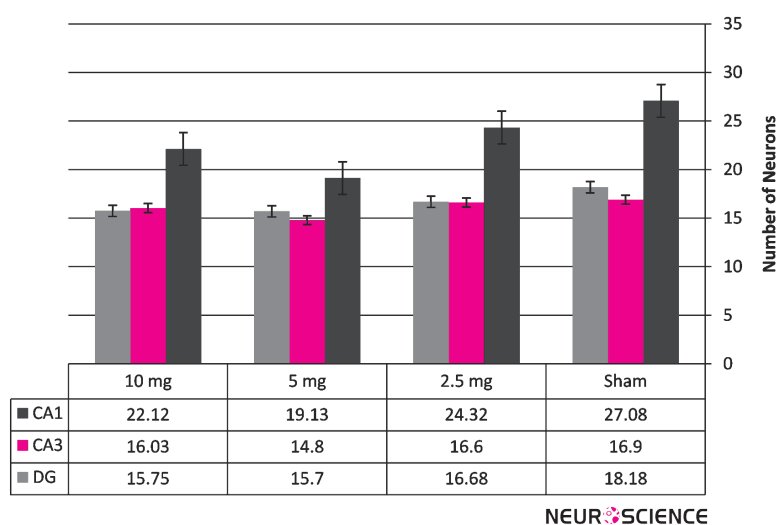


Figure 3. Significant difference in the number of neurons between sham (Control-saline) and MDMA treated groups in all areas of the hippocampus

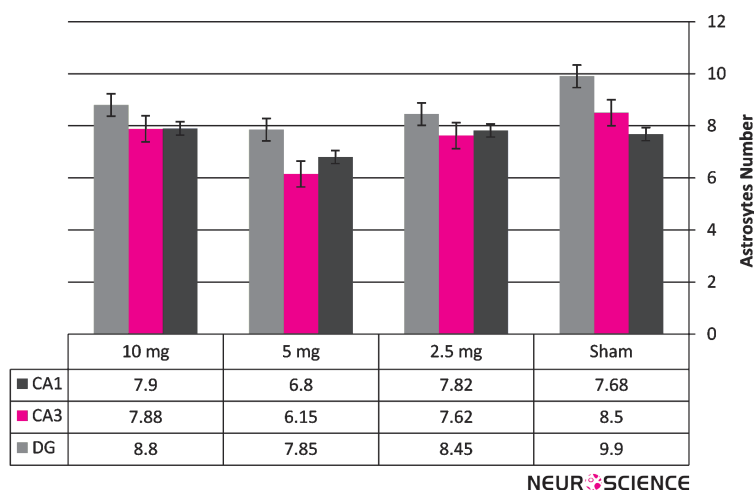


Figure 4. Significant difference in the number of astrocytes between sham (Control-saline) and MDMA treated groups in all areas of the hippocampus

3.2. Cell Counts

Fig.3 shows the results of cell counts (neurons) in different groups. The number of neurons in all experimental groups was lower than in the control-saline group and the differences were significant ($P < 0.05$).

The highest decrease in the number of neurons was shown in response to MDMA with the dose of 5 mg/kg compared to the control-saline, in all regions of the hippocampus.

We found that the number of astrocytes in all areas of hippocampus reduced compared to the sham group (Fig. 4). The highest decrease in astrocytes number was shown in response to 5 mg/kg of MDMA compared to

the control-saline, in all regions of the hippocampus ($P < 0.05$).

4. Discussion

This study surveyed the effects of repeated injection of MDMA on avoidance memory of rats and its effect on hippocampal neurons and astrocytes density. Our results show that the lowest entrance delay in dark chamber was in the control-saline group that received saline, but most delay was shown in the 10 mg/kg MDMA group, probably due to a memory recalling mechanism. It seems that this recalling is not dose-dependence, because the maximum effect of MDMA was seen with 5 mg/kg and not 10 mg/kg. Previous studies demonstrated

that MDMA can change some factors in CNS, for example, a single administration of MDMA (20 mg/kg, i.p) can reduce 5-hydroxytryptamine (5-HT) content and [3H]paroxetine-labeled 5-HT transporter density in the frontal cortex, striatum and hippocampus by 40-60%, 1 week later. It can also increase glial fibrillary acidic protein (GFAP) immunoreactivity in the hippocampus (Aguirre, Barrionuevo, Ramírez, Río, & Lasheras, 1999), whereas we found different results in the number of astrocytes in all areas of the hippocampus.

The hippocampal dentate gyrus generates neurons throughout life and adult hippocampal progenitor cells are located at the subgranular zone between the dentate hilus and the inner margins of the dentate granule cell layer (Fazeli et al., 2008; Jahanshahi et al., 2011). We found that MDMA inhibits neurogenesis in dentate gyrus, because the number of neurons decreased in experimental groups compared to the saline group.

In accordance to our study, Cho et al. (2007) found that oral treatment with MDMA (1.25, 5, 20, 40 mg/kg) or saline for 30 days, dose dependently induced a decrease in the number of BrdU-positive cells in the male and female dentate gyrus. Their results suggest that chronic exposure to MDMA suppresses cell proliferation in the dentate gyrus (Cho et al., 2007).

There are some discrepancies in the effects of MDMA on the neuronal density. For example, some studies indicate that in Alzheimer's disease and other chronic neurodegenerative disorders, MDMA (10–50 mg/kg intraperitoneally) enhances the formation of reactive oxygen species and induces reactive gliosis in the hippocampus, without histological evidence of neuronal loss (Busceti et al., 2008). But other evidence indicates that MDMA significantly enhances neuronal death in the neonatal rat brain. Brain regions mainly affected were the cortex, septum, thalamus, hypothalamus and the CA1 region of hippocampus. These data suggest that a single injection of MDMA (60 mg/kg) causes neurodegeneration in the neonatal rat brain (Dzietko et al., 2010).

In addition, neuron apoptosis induced by MDMA and the expression of apoptosis-related factors in rat brain reported in previous studies, e.g. injection of MDMA intraperitoneally (Wang et al., 2009) or directly intra hippocampus (A. Azami et al., 2009) compared with the saline group, induced apoptosis and significantly increased caspase-3 activity.

5. Conclusion

It can be concluded that MDMA has neurodegenerative effect and it can reduce the density of neurons in hippocampal formation, probably in a dose and duration dependent manner. But it seems that MDMA has various effects on astrocytes density. In addition, long-term administration of MDMA can compensate recalling the passive avoidance memory test compared to the saline group.

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